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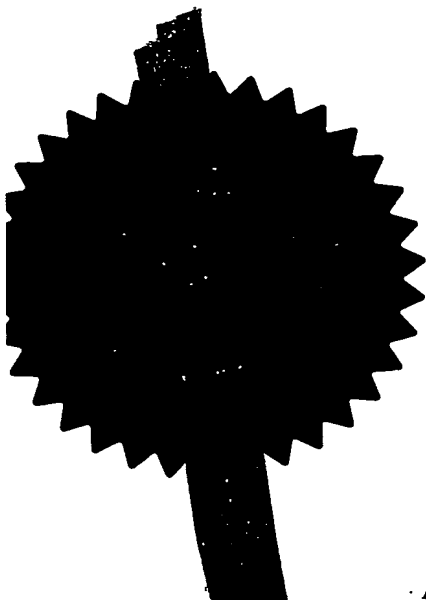
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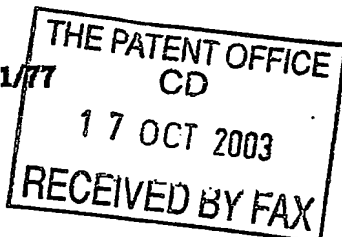
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Dated 8 November 2004



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1. Your reference

P35684-/LMC/GST

2. Patent application number

(The Patent Office will fill in this part)

0324345.8

3. Full name, address and postcode of the or of each applicant (*underline all surnames*)University Court of the University of Edinburgh
Old College
South Bridge
Edinburgh
EH8 9YLPatents ADP number (*if you know it*)

798678001

If the applicant is a corporate body, give the country/state of its incorporation

4. Title of the invention

"Tissue Repair"

5. Name of your agent (*if you have one*)

Murgitroyd & Company

"Address for service" in the United Kingdom to which all correspondence should be sent (*including the postcode*)Scotland House
165-169 Scotland Street
Glasgow
G5 8PLPatents ADP number (*if you know it*)

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6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and (*if you know it*) the or each application number

Country

Priority application number
(*if you know it*)Date of filing
(*day / month / year*)

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application

Date of filing
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Yes

- a) any applicant named in part 3 is not an inventor, or
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11. I/We request the grant of a patent on the basis of this application.

Signature Murgitroyd & Company Date 17 October 2002
Murgitroyd & Company

12. Name and daytime telephone number of person to contact in the United Kingdom

Gordon Stark

0141 307 8400

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I

1 "Tissue Repair"

2

3 The present invention relates to compounds and
4 methods of repairing tissue in diseases where the
5 extracellular matrix is degraded. More particularly
6 the invention relates to compounds including
7 antibodies which increase extracellular matrix
8 anabolism and the use of a novel pathway to find
9 compounds which are capable of use in therapy to
10 increase extracellular matrix anabolism.

11

12 The Extracellular Matrix: Composition and Structure

13

14 The extracellular matrix (ECM) is a complex composite
15 of proteins, glycoproteins and proteoglycans (PGs).
16 Awareness of this complexity has been heightened by
17 the recognition that ECM components, individually or

2

1 in concert with each other or other extracellular
2 molecules, profoundly influence the biology of the
3 cell and hence of the physiology of the whole
4 structure in which the cell is embedded into. The
5 functions of the ECM described so far are many but
6 can be simply categorised as control of cell growth,
7 providing structural support and physical
8 stabilisation, affecting cell differentiation,
9 orchestrating development and tuning metabolic
10 responses (26).

11

12 PGs are a family of heterogeneous and genetically
13 unrelated molecules. The number of full-time as well
14 as part-time members is constantly expanding. The
15 term full-time and part-time refers to the fact that
16 some known PGs can exist as glycoproteins and some
17 proteins can be found in a glycosylated form. In
18 general, PGs are composed of a core protein to which
19 one or more GAG chains are covalently attached by N
20 or O linkage. GAGs are highly anionic linear
21 heteropolysaccharides made of a disaccharide repeat
22 sequences (36). However, there have been reports of
23 PGs devoid of the GAG side chain (3,78). GAGs can be
24 classified into four distinct categories based on
25 their chemical composition (36). The first category
26 is the chondroitin/dermatan sulphate (CS/DS) chain
27 consisting of alternating galactosamine and
28 glucuronic/iduronic acid units. A second class, which
29 is by far the most structurally diverse, is the

3

1 heparin/heparan sulphate (H/HS) group which is
2 composed of alternating glucosamine and
3 glucuronic/iduronic repeats. The third type is the
4 glucosamine and galactose containing keratan sulphate
5 (KS) GAG. Hyaluronic acid (HA) is composed of
6 glucosamine and glucuronic acid repeats. It is the
7 most distinct GAG since it is not sulphated and is
8 not covalently linked to the core protein of PG.
9 Instead, HA binding to the PG core protein is
10 mediated by a class of proteins known as HA binding
11 proteins which exist in the ECM, on the cell surface
12 and intracellularly (68).
13
14 Perlecan is a large HSPG with a core protein size of
15 400-450 kDa known to possess three HS chains. It was
16 first isolated by Hassell et al. (28). It acquired
17 its name from its appearance in rotary shadowing
18 electron microscopy where it looks like a pearl on a
19 string. It is a large multi-domain protein and thus
20 one of the most complex gene products (16,35).
21 Domain I is the N-terminus and contains acidic amino
22 acid residues which facilitate the polymerisation of
23 heparan sulphate (35). However, recombinant domain I
24 has been shown to accept either HS or CS chains; an
25 observation that has been confirmed by *in vitro* study
26 characterising PGs synthesised in response to
27 transforming growth factor β (TGF- β) and fetal calf
28 serum showing that perlecan can be synthesised with
29 CS chains (10). Ettner et al. (17) have shown that

4

1 the ECM glycoprotein laminin binds to perlecan domain
2 I, as well as domain V both of which can carry the HS
3 side chain. Loss of the HS chain abolished the
4 binding.

5

6 Globular domain II was postulated to mediate ligand
7 binding by the low-density lipoprotein (LDL) receptor
8 due to their homology (21,53). Heparitinase treatment
9 abrogates this interaction pointing to the fact that
10 the HS GAG side chains are involved in the binding
11 (21).

12

13 Domain III of perlecan contains an RGD tripeptide
14 sequence that provides a binding capacity for
15 integrin receptors and provides anchorage for the
16 cell (13). Yamagata et al. have shown using double-
17 immunofluorescence that perlecan colocalizes with
18 integrins in cultured fibroblasts (76). This domain
19 has also been shown to be homologous to the laminin
20 short arm (34).

21

22 Domain IV is the largest domain of perlecan
23 containing a series of Ig-like repeats similar to
24 those found in the Ig superfamily of adhesion
25 molecules leading to the speculation that it may
26 function in intermolecular interactions (31).
27 Finally, domain V possessing three globular domains
28 homologous to the long arm of laminin is thought to

5

1 be responsible for self-assembly and laminin mediated
2 cell adhesion (11).

3

4 The multiplicity and variety of perlecan's structural
5 domains are indicative of its potential functions.

6 Perlecan, in addition to binding to laminin and
7 integrins, has been shown to bind fibronectin via its
8 core protein (34). The HS chains of perlecan have

9 also a very important functional role which has
10 proven to be diverse. It has been reported that
11 perlecan mediates the interaction between skeletal
12 muscle cells and collagen IV via the HS GAG side
13 chain (71). Recent studies have led to the
14 identification and characterisation of perlecan as a
15 ligand for L-selectin in the kidney (44). Whether
16 this interaction is via the core protein and/or the
17 HS side chain is not clear. The group of Varki has
18 identified in a series of experiments the HS GAG as
19 well as heparin from endothelial cells as a ligand
20 for both L- and P- selectins but not E-selectins
21 (40,54). The HS side chains in general, and those
22 attached to perlecan core protein in particular, are
23 known to bind growth factors such as fibroblast
24 growth factors (FGF)-2, FGF-7, TGF- β , platelet
25 factor-4 and platelet-derived growth factor-BB (PDGF-
26 BB) (22,35). The functional significance of these
27 interactions has been highlighted by numerous studies
28 demonstrating the role of perlecan in angiogenesis
29 (4,61), the control of smooth muscle cell growth (9)

6

1 and the maturation and maintenance of basement
2 membranes (14). The functional importance of perlecan
3 has been demonstrated by a study of mice lacking
4 perlecan gene expression (14). Homozygous null mice
5 died between embryonic days 10 and 12. The basement
6 membranes normally subjected to increased mechanical
7 stresses such as the myocardium lost their integrity
8 and as a result small clefts formed in the cardiac
9 muscle leading to bleeding in the pericardial sac and
10 cardiac arrest. The homozygotes also had severe
11 cartilage defects characterised by chondrodysplasia
12 despite that fact that it is a tissue which normally
13 lacks basement membrane. This finding was interpreted
14 as a potential proteolysis-protective function for
15 perlecan in cartilage (14). The delay in detecting
16 abnormalities till E10 suggests a certain redundancy
17 with compensatory molecules being able to substitute
18 for perlecan such as the basement membrane HSPGs
19 collagen XVIII (25) and agrin (24).

20
21 Large aggregating PGs are, to date, composed of four
22 members; versican, aggrecan, neurocan and brevican
23 (35). The hallmark of these PGs is the ability to
24 bind hyaluronic acid forming highly hydrated
25 aggregates. They are also characterised by their
26 tridomain structure composed of an N-terminal domain
27 where HA binding occurs, a central domain carrying
28 the GAG side chains and lectin binding C-terminus.

1
2 Versican is a PG with a core protein of 265 - 370 kDa
3 which was originally isolated from human fibroblasts
4 and is the homologue of the avian PG-M (82). It can
5 possess 10-30 chains of CS and has been also reported
6 to carry KS GAG chains (81). It is expressed by
7 keratinocytes, smooth muscle cells of the vessels,
8 brain and mesengial cells of the kidney. The N-
9 terminal domain is responsible for the hyaluronic
10 acid binding properties of versican (42). The central
11 domain of versican consists of the GAG binding
12 subdomains, GAG- α and GAG- β . These subdomains are
13 encoded by two alternatively spliced exons and this
14 gives rise to different versican isoforms. To date
15 four isoforms have been recognised. V0 contains both
16 GAG- α and GAG- β . V1 and V2 are known to possess
17 domain GAG- β and GAG- α respectively (81). V3 is the
18 variant which contains neither of the two subdomains
19 and hence carries no CS/DS GAG side chains and has
20 been localised in various mammalian tissues
21 (43,57,77). The third domain of versican is the C-
22 terminus and consists of a lectin-binding domain, an
23 EGF-like domain and a complement regulatory protein-
24 like domain. This C-terminus binds the ECM
25 glycoprotein, tenascin (2), heparin and heparan
26 sulphate (62) and fibulin (1). Versican is known to
27 have an inhibitory effect on mesenchymal
28 chondrogenesis (80), promotes proliferation (79) and
29 migration via the formation of pericellular matrices

8

1 via its interaction with cell surface bound
2 hyaluronic acid (18). The formation of pericellular
3 matrices is not only achieved via the core protein
4 association with HA but also through GAG side chain
5 interaction with the cytoskeletal associated cell
6 surface receptor, CD44 (37). The postulated role of
7 versican in migration has been also further
8 reinforced by the recent findings of its interaction
9 with both L- and P- selectins via the CS/DS side GAG
10 chains (38). Furthermore, versican GAG side chains
11 modulate chemokine response (30) and has been
12 recently reported to possess growth factor binding
13 capacity (83) and binding to β_1 integrin (75).

14
15 Aggrecan is another large aggregating proteoglycan.
16 It is known to be a major structural component of
17 cartilage. It is composed of three globular domains
18 and two GAG attachment domains (72). The N- terminal
19 globular domain (G1) binds HA and link protein to
20 form large aggregates. The second globular (G2)
21 domain is unique to aggrecan and has no HA binding
22 capacity. The function of this domain has not been
23 clearly defined. The interglobular domain between the
24 G1 and G2 contains proteolytic cleavage sites for
25 metalloproteinases and thus been heavily investigated
26 in pathologies where degradation of this domain is a
27 hallmark, such as osteoarthritis. A KS domain is
28 located at the C-terminus of the G2 domain followed
29 by the CS domain. The CS domain is the largest domain

9

1 of aggrecan and the domain which contributes to the
2 hydrated gel-like forming capacity of aggrecan and
3 thus its importance in load-bearing function. The
4 last domain is the globular domain (G3) which
5 contains three modules: an epidermal growth factor-
6 like domain, a lectin module and a complement
7 regulatory module. This domain is responsible for the
8 interaction of aggrecan with the ECM glycoprotein,
9 tenascin.

10

11 **Functions of Extracellular Matrix Proteoglycans**

12

13 In addition to contributing to the mechanical
14 properties of connective tissues, ECM PGs have
15 biological functions which are achieved via specific
16 classes of surface receptors. The two main,
17 elaborately described, classes are syndecan and
18 integrin receptor families (26). However, other
19 receptors have also been described to bind ECM
20 components such as the selecting family of
21 glycoproteins (54), CD44 with all its variants (23),
22 cell surface enzymes such as hyaluronic acid
23 synthases (63), and PGs (35). It is important not to
24 ignore the fact that the effects of the ECM do not
25 and cannot, in an *in vivo* milieu, ever occur without
26 the influence of other molecules. This statement is
27 based on two well-described concepts. The first being
28 that part of the effects of growth factors,
29 cytokines, hormones and vitamins, as well as cell-to-

10

1 cell contact and physical forces is alteration of the
2 ECM production. The second concept is that the
3 effects of the ECM on the cell bear a striking
4 similarity to those effects observed in response to
5 the above mentioned factors. This is a phenomenon
6 known as "mutual reciprocity" (26) which is an
7 oversimplified view of a complex set of modular
8 interactions, i.e. as defined by Hartwell et al. (27)
9 "cellular functions" carried out by "modules" made up
10 of many species of interacting molecules". The
11 outcome is a summation of all these modules which
12 often interact with each other in a non-vectorial
13 manner.

14
15 Integrins are a family of α, β heterodimeric receptors
16 that mediate dynamic linkages between extracellular
17 adhesion molecules and the intracellular actin
18 cytoskeleton. Although integrins are expressed by all
19 multicellular animals, their diversity varies widely
20 among species (32,33,69). To date 19 α and 8 β subunit
21 genes encode polypeptides that combine to form 25
22 different receptors. Integrins have been the subject
23 of extensive research investigating the molecular and
24 cellular basis of integrin function. Integrins are
25 major contributors to both the maintenance of tissue
26 integrity and the promotion of cellular migration.
27 Integrin-ligand interactions provide physical support
28 for cell cohesion, generation of traction forces in
29 cellular movement, and organise signalling complexes

11

1 to modulate cellular functions such as
2 differentiation and cell fate. PGs are key ECM
3 components which interact with integrins modifying
4 their function and integrins, in turn, are key
5 regulators of ECM PGs. Furthermore, animal-model
6 studies have also shown that integrins contribute to
7 the progression of many common diseases, and thus are
8 implicated as potential drugable targets. The use of
9 anti-integrin monoclonal antibodies and ligand-
10 mimetic peptides has validated this suggestion for
11 inflammatory, neoplastic, traumatic and infectious
12 conditions (15). This potential has lead to attempts
13 to understand more about the mechanisms underlying
14 tissue organisation and cellular trafficking, and to
15 identify approaches for regulating these processes in
16 disease, as well as determining the molecular basis
17 of integrin function. To date the tertiary structure
18 of the integrin dimer is unknown and therefore the
19 current understanding of the molecular basis of
20 integrin function is compiled from the results of a
21 large number of studies which, although indirect,
22 have employed a wide range of complementary
23 technologies.

24

25 Maintenance of the Extracellular Matrix

26

27 ECM homeostasis is maintained under normal
28 physiological conditions by a fine balance between
29 degradation and synthesis orchestrated by matrix

12

1 metalloproteinase (MMPs) and tissue inhibitors of
2 metalloproteinase (TIMPs). This homeostasis is
3 critical in many physiological processes such as
4 embryonic development, bone growth, nerve outgrowth,
5 ovulation, uterine involution, and wound healing.
6 MMPs also have a prominent role in pathological
7 processes such as arthritis (45,49,60), chronic
8 obstructive pulmonary disease (12,66) and
9 atherosclerosis (46). Most of the research to date
10 has focused on elucidating the regulation of these
11 enzymes at the level of transcription, activation of
12 proenzyme, and inhibition by natural inhibitors such
13 as the tissue inhibitors of metalloproteinases.
14 However, little is known about how they are anchored
15 outside the cell.
16
17 Mechanical forces are known to modulate a variety of
18 cell functions such as protein synthesis,
19 proliferation, migration or survival and by doing so
20 regulate tissue structure and function. The routes by
21 which mechanical forces influence cell activities
22 have been defined as mechanotransduction and include
23 the tensegrity structure model and signalling through
24 cell surface mechanoreceptors including ECM binding
25 molecules. The tensegrity structure model postulates
26 that a cell maintains a level of pre-stress generated
27 actively by the actin microfilaments and intermediate
28 filaments (48). This active stress element is
29 balanced by structures resisting compression, mainly

13

1 microtubules within the cell and components of the
2 ECM. Matrix remodelling in response to mechanical
3 forces is an adaptive response to maintain tensegrity
4 in mechanosensitive tissues including cartilage and
5 lung. *In vivo* and *in vitro* observations demonstrate
6 that mechanical stimulation is necessary to maintain
7 optimal cartilage and lung structure and function
8 (50,55,55,64,74). Thus mechanical forces regulate ECM
9 composition which, in turn, will modify the
10 mechanical microenvironment in tissues in a mutually
11 reciprocal manner. This aspect provides a valuable
12 tool for investigating biological functions *in vitro*.

13

14 Extracellular Matrix Catabolism and Anabolism

15

16 Cartilage is specialised connective tissue with a
17 major function to bear mechanical stress without
18 permanent distortion, acting as a shock absorber for
19 joints. The ECM, accounting for 90% of the dry weight
20 of cartilage consists primarily of large, hydrated PG
21 aggregates trapped within a mesh of collagen fibrils.
22 The ECM provides the tensile strength of cartilage as
23 well as affecting cell behaviour.

24

25 Osteoarthritis (OA) is a pathological condition
26 involving progressive degeneration of articular
27 cartilage, remodelling of the sub-chondral bone
28 accompanied by limited synovitis. Treatment of OA
29 could be based on the emerging picture of

14

1 pathophysiological events that govern the initiation
2 and progression of OA. The fundamental event leading
3 to matrix destruction of articular cartilage in OA
4 arises from an imbalance between anabolic and
5 catabolic pathways (19,59). The ECM is degraded by
6 matrix MMPs. Inhibition of the MMPs, their
7 activators, and other factors that induce MMP gene
8 up-regulation or activation is potentially a target
9 for drug development in the treatment of OA (47).
10 This is a novel modality of OA treatment which has
11 focused in the past primarily on the administration
12 of anti-inflammatory drugs such as corticosteroids
13 and non-steroidal anti-inflammatory drugs (NSAIDs)
14 which provide the patient with symptomatic relief
15 from pain and swelling of synovial joints without
16 affecting disease progression (i.e. cartilage
17 degradation and osteophyte formation). Therefore, the
18 statement of Hunter that, "ulcerated cartilage is a
19 troublesome thing; once destroyed is not repaired"
20 remains true to this day (47).
21
22 Novel therapeutic approaches include the application
23 of tissue engineering to the repair of articular
24 surface defects in OA or after injury which will
25 require that viable cells be transplanted to the
26 injured tissue site. This remains a challenging task
27 since the long-term durability of this treatment
28 approach remains in doubt. Genetic engineering offers
29 another therapeutic approach to the regeneration of

15

1 degenerative articular cartilage. The objective is to
2 control the biological systems which are mediating
3 the destruction of ECM in OA. One of the major
4 impediments in this area, however, is which genes to
5 use and how and for how long can they be transferred
6 safely (29,47,65,67).

7

8 Another organ system involving ECM is the lung where
9 it provides structural support and acts as adhesive
10 as well as a guiding cue for diverse biological
11 processes. Collagens are the most abundant ECM
12 component in the lung constituting 60-70 % of lung
13 interstitium followed by elastin and PGs and
14 glycoproteins (70).

15

16 Chronic Obstructive Pulmonary Disease (COPD) is
17 another disease in whose progression ECM plays a
18 major role. COPD is a major cause of chronic
19 morbidity and mortality throughout the world.
20 According to the latest statistics by the World
21 Health Organisation (WHO), COPD is the fourth leading
22 cause of death in the world, and further increases in
23 its prevalence and mortality are predicted in the
24 coming decades. Recently a Global Initiative for
25 Chronic Obstructive Lung Disease (GOLD) was
26 established in collaboration with the US National
27 Heart, Lung, and Blood Institute (NHLBI) and the WHO.
28 According to GOLD, "COPD is a disease state
29 characterised by airflow limitation that is not fully

1 reversible. The airflow limitation is usually both
2 progressive and associated with an abnormal
3 inflammatory response of the lungs to noxious
4 particles or gases". The major symptoms of COPD are
5 cough, sputum production, and dyspnea upon exertion.
6 Of these symptoms, the first to arise is chronic
7 cough and sputum production followed by the
8 development of airflow limitation many years later
9 which could identify individuals at risk of
10 developing COPD. COPD can coexist with asthma, the
11 other major chronic obstructive airway disease
12 characterised by an underlying airway inflammation.
13 However, the inflammation characteristic of COPD is
14 thought to be distinct from that of asthma and the
15 airflow limitation in asthma is far more reversible
16 than that featured in COPD.

17
18 Although the main risk factor for COPD is tobacco
19 smoking, other predisposing factors have been
20 identified. The main cause of chronic airflow
21 limitation in COPD is small airway disease
22 (obstructive bronchiolitis) and parenchymal
23 destruction (emphysema). Chronic inflammation leads
24 to small airway remodelling and subsequent narrowing.
25 Destruction of the lung parenchyma, also by
26 inflammatory processes, leads to the loss of alveolar
27 attachments to the small airways and decreases lung
28 elastic recoil. Based on mechanical interdependence,
29 the loss of alveolar attachment also contributes to

17

1 the diminished ability of the airways to remain open
2 during expiration. Thus, the major processes known to
3 play a key role in the pathogenesis of COPD are
4 inflammation, an imbalance of proteinases and
5 antiproteinases in the lung, and oxidative stress
6 which leads to the destruction of the ECM (56).

7

8 To date, the only available drug treatments for COPD
9 sufferers focus primarily on bronchodilation using
10 anticholinergics and dual β 2-dopamine₂ receptor
11 antagonists. Since the inflammation in COPD is
12 resistant to corticosteroids, much anticipation is
13 put into novel anti-inflammatory agents currently in
14 development which include phosphodiesterase
15 inhibitors, nuclear factor κ B inhibitors and p38 MAP
16 kinase inhibitors. Matrix metalloproteinase (MMP)
17 inhibitors are also currently being developed
18 although in their current formulation they have
19 serious toxicity side effects. However, what is
20 lacking so far is an agent which may aid in the
21 repair of injured ECM (6-8).

22

23 In summary, both disease paradigms have a strong
24 element of ECM remodelling as a major contributor in
25 their pathophysiology. Furthermore, current
26 therapeutics have focused primarily on preventative
27 or symptom-relieving treatments. However, due to the
28 progressive nature of both diseases together with

18

1 often late diagnosis, regaining normal function

2 remains a problem.

3

4 Recently, novel therapeutic approaches targeting

5 integrin function have been adopted. Very late

6 antigen-4 (VLA4) or $\alpha 4$ integrin antagonists are

7 currently in advance stages of trials for the

8 treatment of asthma (39,41,51). In cartilage,

9 antagonists to $\alpha v \beta 3$ integrin have attenuated

10 adjuvant-induced arthritis and now are undergoing

11 trials (5). However, in both cases the target of the

12 functional blocking is attenuating inflammation and

13 this has not been demonstrated to affect the ECM

14 alteration usually associated with those conditions.

15

16 Accordingly, it is an object of the present invention

17 to provide a compound for use in ECM anabolism. It

18 is a further aim of the present invention to provide

19 a technique to screen compounds for use in ECM

20 anabolism.

21

22 According to the present invention there is provided

23 a compound for use in tissue repair wherein the

24 compound modifies the function of $\beta 1$ integrin.

25

26 Modification includes a change in the function of ,

27 or the inhibition of the binding of, or the shedding

28 of the $\beta 1$ integrin.

29

19

1 Preferably the compound is an inhibitor of the $\beta 1$
2 integrin.

3
4 More preferably the compound functionally blocks
5 $\beta 1$ integrin.

6
7 Most preferably the compound binds the molecule in
8 the region of amino acid residues 82-87 of
9 $\beta 1$ integrin.

10
11 In the known sequence, residues 82 to 87 are
12 considered to be the residues of the sequence nprgsk
13 (Asparagine-Proline-Arginine-Glycine-Serine-Lysine).

14
15 In one embodiment the compound is a synthetic
16 peptide.

17
18 In a further embodiment the compound is an antibody.

19
20 The antibody should preferably be a humanised
21 antibody.

22
23 Alternatively the antibody could be a chimeric
24 antibody.

25
26 In one embodiment the antibody could be based on or
27 derived from the functional modifying antibody of
28 $\beta 1$ integrin obtainable as a commercial clone JB1a from
29 Chemicon.

1
2 The term antibody describes an immunoglobulin (Ig)
3 molecule which is naturally synthesised or
4 alternatively which is partly or wholly synthetically
5 produced. Further, the term also includes any
6 natural protein or partly or wholly synthetically
7 produced protein having a binding domain which is, or
8 is substantially homologous to an immunoglobulin
9 binding site.

10
11 The invention also provides a method to screen for
12 compounds for use in tissue repair wherein the method
13 comprises the step of determining the ability of a
14 compound to modify the function of the $\beta 1$ integrin.

15
16 Modification includes a change in the function of ,
17 or the inhibition of the binding of, or the shedding
18 of the $\beta 1$ integrin.

19
20 Preferably the method comprises the step of
21 determining the ability of a compound to bind the
22 domain corresponding to residues 82-87 of $\beta 1$ integrin
23 (residues nprgsk (Asparagine-Proline-Arginine-
24 Glycine-Serine-Lysine)).

25
26 The invention further provides a compound identified
27 from the method described herein.

28

21

1 The invention further provides a medicament suitable
2 for use in tissue repair wherein the medicament
3 includes as an active ingredient, a compound which
4 modifies the function of $\beta 1$ integrin.
5 Preferably the compound binds amino acid residues 82-
6 87 of $\beta 1$ integrin (residues nprgsk (Asparagine-
7 Proline-Arginine-Glycine-Serine-Lysine)).

8
9 The medicament may be administered together with any
10 suitable carrier.

11
12 The invention further provides the use of an antibody
13 to $\beta 1$ integrin in the preparation of a medicament for
14 treatment of injured tissue.

15
16 The invention further provides the use of the
17 sequence of amino acids at positions 82-87 of $\beta 1$
18 integrin in the identification of a compound to
19 promote tissue repair by increasing ECM anabolism.

20
21 The sequence of amino acids 82-87 is nprgsk
22 (Asparagine-Proline-Arginine-Glycine-Serine-Lysine).

23
24 In a yet further embodiment of the present invention,
25 there is provided a compound which causes the
26 shedding of the $\beta 1$ integrin.

27

1 Preferably the compound binds amino acid residues 82-
2 87 of β 1 integrin.

3
4 In the known sequence, residues 82-87 are considered
5 to be the residues of the sequence nprgsk
6 (Asparagine-Proline-Arginine-Glycine-Serine-Lysine).

7
8 In one embodiment the compound is a synthetic
9 peptide.

10
11 In a further embodiment the compound is an antibody.

12
13 The antibody should preferably be a humanised
14 antibody.

15
16 Alternatively the antibody could be a chimeric
17 antibody.

18
19 It is thought that a compound according to the
20 present invention may act by shedding the β 1
21 integrin.

22
23 Substitutions may be made to the binding epitope as
24 defined in the present invention, for example amino
25 acid residues may be substituted with a residues of
26 the same or similar chemical class, and which result
27 in no substantial conformational change of the
28 binding epitope.

29

23

1 In a yet further embodiment of the present invention,
2 there is provided a compound for use in tissue repair
3 wherein the compound binds to any nature-similar or
4 mimetic molecule which has conformational homology to
5 the $\beta 1$ integrin. In other words the three-
6 dimensional shape of the mimetic molecule is
7 substantially super-imposable upon the three-
8 dimensional shape of the $\beta 1$ integrin.

9
10 Preferably the nature-similar or mimetic molecule has
11 a conformational homology to amino acid residues 82
12 to 87 of the $\beta 1$ integrin.

13
14 Residues 82 to 87 are known to have the sequence
15 nprgsk (Asparagine-Proline-Arginine-Glycine-Serine-
16 Lysine).

17
18 In a preliminary experiment, the present inventors
19 attempted to investigate the role of the cell surface
20 receptors in the synthesis of ECM which are altered
21 in diseases such as COPD and OA and are important for
22 lung and cartilage function microscopically and
23 macroscopically.

24
25 The results described herein demonstrate that
26 functional modification of $\beta 1$ integrin through a
27 domain corresponding to amino acid residues 82-87
28 induces a substantial time- and dose- dependent
29 increase in ECM in normal, osteoarthritic and lung

1 epithelial cells in monolayer and 3-dimensional
2 cultures. The 3-dimensional alginate culture prevents
3 dedifferentiation of chondrocytes and hence the loss
4 of chondrocytic phenotype. This inhibitory domain is
5 different from those previously described which bind
6 to amino acid sequence with residues 207-218 (20). It
7 is also distinct from the known stimulatory domains
8 which are localised to those amino acid residues and
9 residues 657-670 and 671-703 (73). Modulation of the
10 cytokine TGF- β induced a less profound increase which
11 was also time- and dose-dependent. This increase in
12 all ECM PGs was sustained for extended periods of
13 time without any additive doses.

14
15 These experiments demonstrate a novel finding which
16 is that an increase in ECM can be achieved via the
17 modulation of cell surface receptors and to a much
18 lesser extent by modulating the binding of a soluble
19 factor in a time- and dose-dependent manner in both
20 pulmonary and cartilage derived cells. When performed
21 in conjunction with mechanical stimulation, the ECM
22 increase demonstrated altered organisation which
23 could signify functionality of the PGs. One
24 potential, but non-binding mechanistic hypothesis is
25 that this modulation may have led to alteration in
26 the proteinase/antiproteinase balance which can be
27 sequestered onto the surface of cells since the
28 response was so rapid that no gene transcription or
29 translation could have occurred.

25

1
2 The potential of these findings lie in tissue repair
3 in disease where the matrix is degraded and cannot be
4 replenished as in diseases like COPD and OA. The
5 finding may offer a venue for therapeutic
6 intervention in such diseases where the only current
7 lines of therapy focus on alleviating the symptoms by
8 the use of anti-inflammatory agents but has no
9 potential for regaining function. This could be
10 achieved via the administration of humanised or
11 chimeric antibodies or synthetic peptides capable of
12 binding the specific region of the $\beta 1$ integrin. In
13 summary, the results herein address a different
14 potential therapeutic modality which focuses on
15 increasing ECM anabolism instead of decreasing
16 catabolism.

17
18 The invention is exemplified herein with reference to
19 the following non limiting examples and with
20 reference to the figures.

21
22 In the figures:

23
24 Figure 1 illustrates time- and dose-dependent
25 effects of functional modifying antibody of $\beta 1$
26 integrin and TGF- β on proteoglycans,

27

26

1 Figure 2 illustrates the effect of $\beta 1$ integrin
2 functional modification in combination with
3 ~~mechanical stimulation of monolayer cultures,~~

4
5 Figure 3 illustrates the effect of $\beta 1$ integrin
6 and TGFB blocking in combination with
7 mechanical stimulation of normal chondrocytes,

8
9 Figure 4 illustrates the effect of $\beta 1$ integrin
10 functional modification and TGF- β blocking in
11 combination with mechanical stimulation on
12 osteoarthritic chondrocytes,

13
14 Figure 5 illustrates PG content in the
15 alginate cultures in response to functional
16 modification of $\beta 1$ integrin and neutralising
17 TGF- β in relation to time and dose,

18
19 Figure 6 illustrates dose- and time-dependent
20 effects of functional modification of $\beta 1$
21 integrin and neutralising TGF- β on ECM PG from
22 H441 cell lines, and

23
24 Figure 7 shows the amino acid sequence of $\beta 1$
25 integrin which is nprgsk (Asparagine-Proline-
26 Arginine-Glycine-Serine-Lysine), and

27

27

1 Figure 8 shows the presence of a 110kDa $\beta 1$
2 integrin in the media of chondrocytes in
3 alginate cultures and H441 cells separated
4 onto 6% SDS-polyacrylamide gels following $\beta 1$
5 integrin function modulation but not following
6 TGF- β neutralisation.
7

8 Stimulation Protocol and Extraction Procedure

9
10 Primary human chondrocytes from articular knee joints
11 were obtained with consent and cultured in either
12 monolayers (5×10^4 /ml) or embedded into 1% alginate
13 (1:1 v/v of Keltone and Pronova) at density of 5×10^4
14 and 50×10^4 /ml respectively in Iscoves media (Sigma
15 Aldrich) and maintained at 5% in an CO₂ incubator.
16 Cultures were subjected to serum starving overnight
17 in a medium containing 0.5% foetal calf serum.
18 Functional modifying antibody of $\beta 1$ integrin
19 (Chemicon, clone JB1a) was added to the cultures at
20 concentration of 1.44 and 0.48 μ g/ml. The $\beta 1$ integrin
21 stimulatory antibody TS2/16 which binds the amino
22 acid region 207-218 was also added at 0.9 μ g/ml for 1
23 hour to demonstrate the specificity of the JB1a
24 action. TGF β neutralising antibody (R&D systems,
25 clone 1D11) was added at a concentration of 0.1 and
26 0.3 μ g/ml where at the lower concentration it
27 neutralises TGF β isoforms 1 and 3 and isoform 2 at
28 the higher concentration. After antibody addition to

28

1 the cells in culture, the medium was aspirated and
2 the cell layer rinsed twice with ice-cold PBS
3 (calcium- and magnesium-free). The media was
4 aspirated and preserved after the addition of
5 protease inhibitors at -80°C . PGs were extracted from
6 the cell layer by extraction buffer containing
7 protease inhibitors (4M guanidium-HCl, 4% (w/v)
8 CHAPS, 100mM sodium acetate buffer at pH 5.8

9 containing protease inhibitors) for 24 hours at 4°C .
10 Monolayers were subjected to mechanical stimulation
11 4000μ strain at 0.33 Hz for 20 min after the addition
12 of the antibodies (30 min) or PBS and collected for
13 extraction after one hour.

14
15 Adenocarcinoma cell line derived from the lung were
16 also used (H441) to test the effect of the antibodies
17 on matrix synthesis. This cell line has epithelial
18 type II characteristics.

19
20 The total protein concentration was estimated using
21 the Bradford method.

22
23 Sample Preparation for Composite Polyacrylamide-
24 Agarose Gel Electrophoresis

25
26 The extracts were precipitated overnight with 9 v/v
27 ethanol at -20°C , centrifuged at 12,000 rpm for 40min
28 at 4°C then resuspended in 0.5M sodium acetate (pH
29 7.3) and precipitated again with ethanol overnight

29

1 and centrifuged. Samples were resuspended in 0.5% SDS
2 and mixed with 1:1 v/v with 50%w/w sucrose in 10mM
3 Tris-HCl (pH 6.8), 0.5% SDS and 0.05% bromophenol
4 blue. 20ug of protein was used for gel loading.

5

6 Gel electrophoresis

7

8 Composite gels (1.5mm thick) containing 0.6% agarose
9 and 1.2% polyacrylamide in Tris-sodium acetate buffer
10 (10mM, pH 6.8) containing 0.25mM sodium sulphate were
11 used for the separation of large PG, versican,
12 aggrecan and perlecan, under associative conditions
13 according to the method of Carney.

14

15 After electrophoretic separation, the samples were
16 transferred onto Hybond ECL-nitrocellulose membrane
17 (Amersham Pharmacia) using a wet blotting unit
18 (BioRad). Membranes were blocked with 5% Milk in TBS
19 pH 7.4 containing 0.1 % v/v Tween-20 and 0.1% sodium
20 azide for 1 hours at room temperature and then
21 incubated with primary antibodies diluted in TBS-
22 Tween 20 for 1 hour at room temperature or overnight
23 at 4°C. The primary antibody for versican (12C5) was
24 mouse anti-human at 1/500 dilution (Hybridoma Bank,
25 Iowa City, Iowa). This antibody recognises the
26 hyaluronic acid binding domain of versican (58).
27 Aggrecan antibody was used at dilution of 1/500
28 aggrecan (Serotec, HAG7E1). Due to the fact that the
29 exact epitope recognised by this antibody is unknown,

1 additional antibodies were used. An antibody
2 recognising amino acid sequence 1-14 in the
3 ~~hyaluronic acid region was used~~ at 1/500 dilution
4 (Biogenesis, Germany). The anti-interglobular domain
5 region antibody 6B4 was also used to confirm the
6 identity of aggrecan (gift from Prof. Bruce Caterson,
7 Cardiff). Perlecan antibody was use at a dilution of
8 1/1000 (7B5, Zymed Laboratories). This antibody has
9 been demonstrated to be immunoreactive to non-
10 degraded forms of perlecan (52). A biotinylated
11 secondary antibody (goat anti mouse Ig, Dako) was
12 added, followed by a 1 hour incubation with 1/1000
13 streptavidin-horseradish peroxidase (HRP) complex in
14 TBS-T. Signal was visualised using the ECLplus
15 (enhanced chemiluminescence) assay (Amersham
16 Pharmacia).

17
18 The same analyses as detailed above were performed
19 using extracts subjected to pre-clearing of the
20 functional modifying antibodies by
21 immunoprecipitation using protein A sepharose
22 according to manufacturer's instructions (Amersham
23 Pharmacia).

24
25 Our experiments demonstrate a novel finding which is
26 that the an increase in ECM PGs anabolism can be
27 achieved via functional modification of the cell
28 surface $\beta 1$ integrin and to a much lesser extent to
29 neutralising TGF β in both time- and dose-dependent

31

1 manner in primary chondrocytes derived from normal
2 and osteoarthritic articular cartilage as well as
3 pulmonary derived cells. This response is also
4 achieved in non-dedifferentiated chondrocyte cultures
5 maintained in alginate matrix. One potential
6 mechanistic hypothesis is that the functional
7 modification of integrin may have led to increased
8 release of tissue inhibitors of MMP (TIMP2) which are
9 known to be sequestered onto the surface of cells by
10 various cell surface molecules such as membrane type-
11 1 MMP. TIMP2 is an inhibitor of MMP2. MMP2 could lead
12 to matrix PG degradation either directly or via
13 inducing MMP9. Thus modifying integrin could have led
14 to inactivation of MMP2 and subsequently MMP9 leading
15 to increased matrix PG accumulation. However, this
16 could be one mechanism and the involvement of another
17 pathway could not be excluded. As to the less
18 profound effect of neutralising TGF β , the answer could
19 lie in the known interaction of MMP9 with TGF β where
20 it has been shown that MMP9 can activate latent TGF β ,
21 rendering it biologically active.

22

23 Although the present invention has been particularly
24 shown and described with reference to a particular
25 example, it will be understood by those skilled in
26 the art that various changes in the form and details
27 may be made therein without departing from the scope
28 of the present invention.

29

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2

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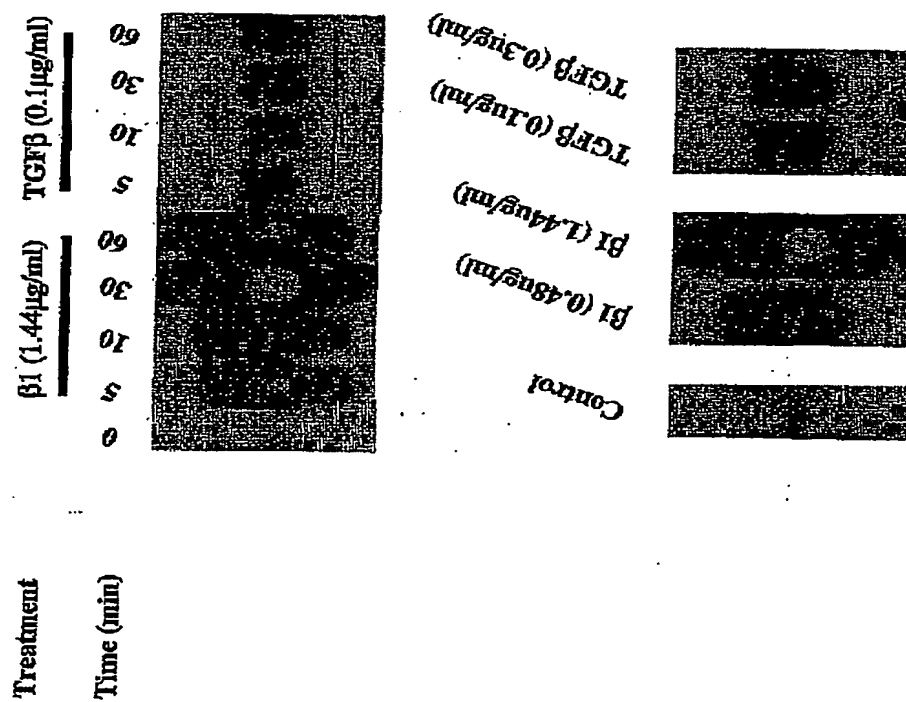


Figure 1.

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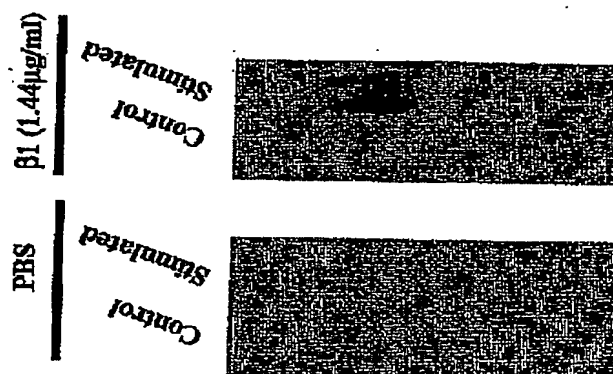


Figure 2.

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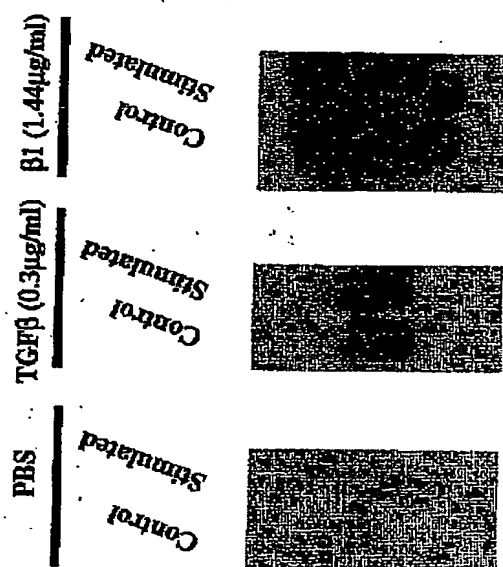
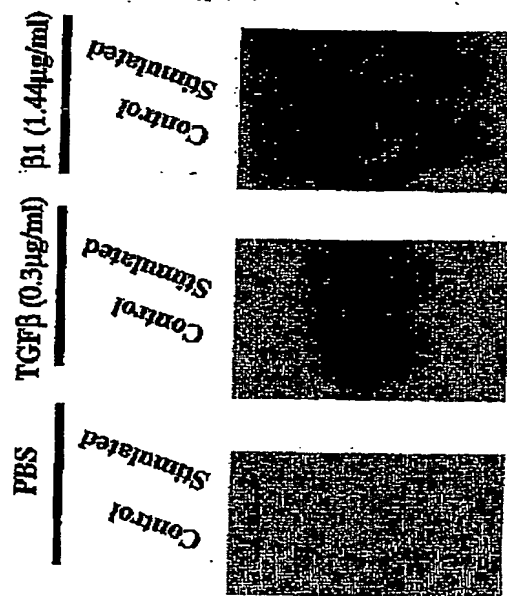


Figure 3.

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Figure 4.

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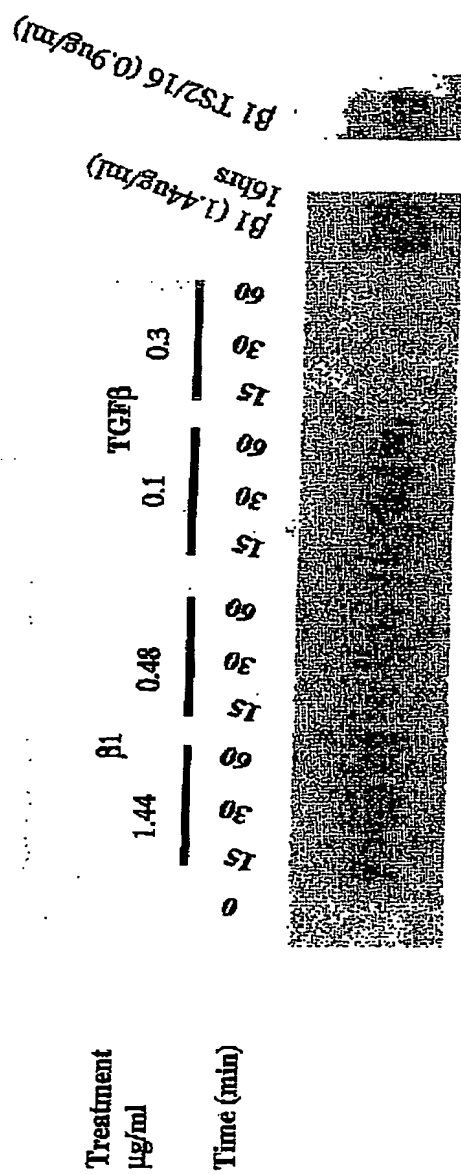


Figure 5.

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Treatment	Time (min)
$\beta 1$ (1.44 $\mu\text{g/ml}$)	0
	5
	10
	30
	60
TGFB (0.3 $\mu\text{g/ml}$)	0
	5
	10
	30
	60

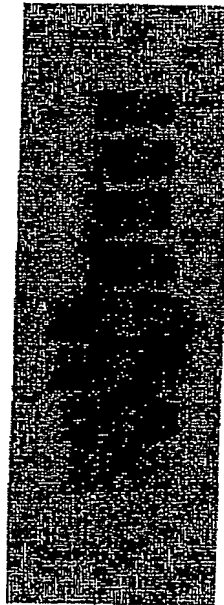


Figure 6.

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Figure 7. - Amino Acid Sequence of beta - 1 integrin

MNLPQIFWIGLISSVCCVFAQTDENRCLKANAKSCGECIQAGPNC
GWCNTNSTFLQEGMPTSAACDDLEALKKKGCPDDIENPRGSKDKKNKNVTNRSGTAE
KLKPEDIHQIQPQQQLVLRRSQEPQTFILKFKRAEDYPIDLYTMDLSYMKDDLENVK
SLGTDLMNEMRRITSDFRIGFGSFVEKTVMPYISTTPAKLRNPCTSEQNCTTPFSYKNV
LSLTNKGEVFNELVGKQRISGNLDSFEGGFDAIMQVAVCGSLIGWRNVTRLLVFSTDAG
PHFAGDGKLGIVLPNDGQCHLENNMYTMSHYDYDYPISIAHLVQKLENNIQTFPAVTEE
FQPVYKBLKNLIPKSAVGTLSANSSNVQLIDAYNSLSSEVILENGKLESEGVTSISK
YCKNGVNGTGNGRKCNSISIGDEVQFHSITSNKCCKKDSDFKTRPLGFTTEEVEVIL
QYICECEBCQSEGIPEPKCHEGNGTFECGACRCNEGRVGRHCECSTDEVNSEDMDAYCR
KENSSEICSNNGECVCGQCVCRRKRDNTNEIYSGKFCECDNFNCDRSNGLICGGNGVCKC
RVCECNPNYTGSA CDCSLDTSTCEASNGQICNGRGICEGVCKCTDPKFGGQICEMCQT
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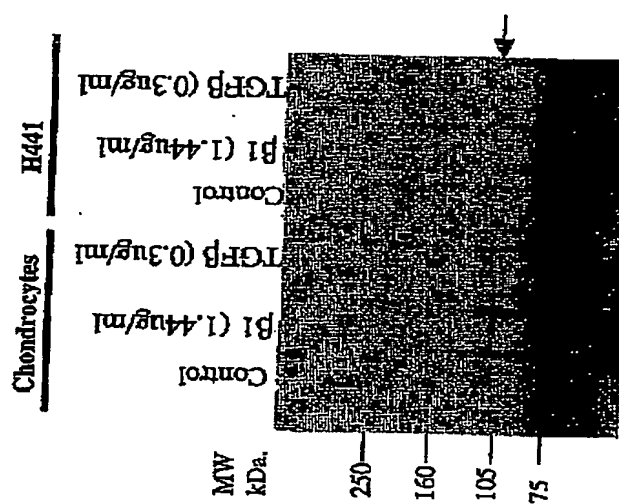


Figure 8.